Root Elongation of Soybean Genotypes in Response to Acidity Constraints in a Subsurface Solution Compartment

Armando Ferrufino, T. Jot Smyth,* Daniel W. Israel, and Thomas E. Carter, Jr.

ABSTRACT

Aluminum-tolerant germplasm is needed to overcome subsurface acidity constraints to root growth and plant access to water and nutrients. Root elongation of four soybean [*Glycine max* (L.) Merr.] genotypes exposed to varying concentrations of Al, H, and Ca were compared in two experiments using a vertically split root system. Roots extending from a limed surface soil compartment grew for 12 d into a subsurface compartment with nutrient solution treatments. In Exp. 1 root growth for cv. Ransom and Plant Introduction 416937 (PI) were compared in solutions with factorial combinations of pH (4.2, 5.2) and Al (0, 7.5, 15 μ*M*) with Ca maintained at 10 m*M*. In Exp. 2 soybean line N93-S-179 (N93), PI, and cultivars Ransom and Young were compared in solutions with factorial combinations of Ca (2 and 10 m*M*) and Al (7.5 and 15 μ*M*) maintained at pH 4.6. Ransom and PI had similar responses in tap and lateral root elongation to solution pH and Al treatments in Exp. 1, but mean tap root length of Ransom in the subsurface compartment exceeded that of PI by 22%. Aluminum inhibited the length of lateral roots more than tap roots in both experiments. Molar activity ratios between Ca and Al^{3+} {Ca/Al^{3+}} accounted for most of the differences in root elongation response among solution treatments in Exp. 2. A 50% reduction in relative length of tap roots for all genotypes occurred with a {Ca/Al^{3+}} value of 891. Values of {Ca/Al^{3+}} for 50% reductions in relative length of lateral roots differed among genotypes and were 1.6 to 3.5 times greater than for tap roots. On the basis of the {Ca/Al^{3+}} indices for lateral root length, line N93 and Ransom exhibited greater tolerance to subsurface solution Al than PI and Young.

Development of soybean roots in acid soils is restricted by low pH, low contents of Ca and Mg, and high Al (Goldman et al., 1989; Carter and Rufty, 1993; Spehar, 1994). Liming alleviates constraints of soil acidity in surface layers (Dierolf et al., 1997), but subsurface layers may remain acid and can have low Ca concentrations (Ritchey et al., 1980). Subsoil acidity reduces crop yields by restricting root growth and thereby reducing nutrient acquisition and plant access to soil water reserves during periods of drought stress. Potential alternatives to the direct amelioration of subsoil acidity include the use of Al-tolerant germplasm (Foy, 1988).

Aluminum rhizotoxicity for several plant species can be alleviated by increasing the Ca concentration in nutrient solutions (Alva et al., 1986a, 1986b; Horst, 1987; Runge and Rode, 1991). Calcium additions alleviate Al rhizotoxicity by reducing Al activity through increased solution ionic strength and increased competition between Ca and Al at binding sites external to the plasmalema (Kinraide and Parker, 1987). Other roles for Ca include increasing membrane hydrophobicity; preventing cell leakiness; strengthened cell walls; and, in the cytoplasm, acting as a "second messenger" that mediates changes in enzyme activity (Hanson, 1984; Marschner, 1995).

Different classes of roots for the same genotype can exhibit differential elongation responses to rhizotoxic Al. Bushamuka and Zobel (1998), in a comparison of elongation of tap, basal, and lateral roots between limed and unlimed subsurface layers of Al-toxic soil among several maize (*Zea mays* L.) and soybean cultivars, found that tolerance to Al in the unlimed subsurface layer by all three root classes was only observed in one maize cultivar. However, among the other maize and soybean cultivars, either one, two, or none of these root classes were tolerant to the soil Al stress. Sanzonowicz et al. (1998a, 1998b) found that subsurface solution H and Al inhibited the length of lateral roots more than tap roots for Ransom soybean. When exposed to similar levels of toxic H or Al, a higher solution Ca concentration was needed to increase length of lateral roots relative to tap roots.

The objective of this investigation was to compare root elongation response to varying solution concentrations of H and Al and the ameliorative effects of Ca among different classes of roots for selected soybean genotypes exposed to rhizotoxic treatments in the subsurface compartment of a vertically split root system.

MATERIALS AND METHODS

A vertically split root system (Sanzonowicz et al., 1998a) was used to evaluate elongation of soybean roots extending from a limed surface soil into a subsurface zone containing solutions with various combinations of Al, pH, and Ca. Plastic tubes, 52 cm long with an internal diameter of 10 cm, were divided into two sections separated by a root-permeable membrane. The membrane was formed by dipping cheesecloth attached to the surface compartment into a mixture of paraffin and petrolatum (1:2) at 80°C for 10 s. Both tube sections were disinfected with 20.6 *M* ethanol.

The 12-cm-long surface compartment was filled with 1.1 kg of sterilized (121°C, 0.1 MPa for 1 h) loamy sand from the Ap horizon of a Wagram soil (loamy, kaolinitic, thermic Arenic Kandiudult). Selected chemical properties of this soil are de-

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Abbreviations: Al_a, fraction of Al in solution that reacts immediately with the ferron reagent; Al_b, fraction of Al in solution that reacts with ferron according to first order kinetics; Al_T, all Al in solution that reacts with ferron; $AlFN_{30}$, Al in solution reacting with ferron by 30 s; Al_{Mono}, $Al^{3+} + Al(OH)^{2+} + Al(OH)_2^+ + Al(OH)_3^0 + Al(OH)_4^-$; {Ca/$Al^{3+}$}, molar activity ratio between Ca and trivalent Al; N93, breeding line N93-S-179; PI, Plant Introduction 416937.

scribed elsewhere (Sanzonowicz et al., 1998a). The soil was limed to pH 5.5 with $CaCO_3$ and received 30 mg K kg^{-1} as KCl before sterilization. The subsurface compartment, which was used for treatment solutions, contained 3.0 L of solution continuously aerated with air prefiltered through a high efficiency particulate filter. Solutions in all experiments contained 18.5 μM B as H_3BO_3 and 0.5 μM Zn as $ZnCl_2$, since root elongation of Ransom decreased in the subsurface compartment when these nutrients were omitted (Sanzonowicz et al., 1998a). Aluminum was supplied as $AlCl_3$ from a 0.037 *M* acidified stock solution and Ca as $CaCl_2$. Solution pH was adjusted daily by titration with 0.05 *M* solutions of either HCl or NaOH. Solutions were replaced every 4 d.

Experiment 1 was conducted during October in a greenhouse at North Carolina State University in Raleigh, NC. Natural light was supplemented by 150 μmol m^{-2} s^{-1} of photosynthetically active radiation from metal halide lamps for 16 h d^{-1}. Treatments in the subsurface solution compartment consisted of a factorial combination of three Al concentrations (0, 7.5, and 15 μM) and two pH levels (4.2 and 5.2) with a constant supply of Ca (10 m*M*). Root elongation into these subsurface solution treatments was compared between soybean plant introduction 416937 (PI), which is part of the USDA plant germplasm collection, and Ransom (Brim and Elledge, 1973).

Experiment 2 was conducted in a walk-in chamber of the phytotron at North Carolina State University (Thomas and Downs, 1991) with day/night temperatures of 26/22°C, 70% relative humidity, and a 12-h photoperiod. Light was provided by incandescent and fluorescent lamps at a photosynthetic photon flux density of 639 mol m^{-2} s^{-1}. Treatments in the subsurface solution compartment consisted of a factorial combination of two Al concentrations (7.5 and 15 μM) and two Ca concentrations (2 and 10 m*M*) maintained at pH 4.6. Root elongation into these solution treatments was compared among four soybean genotypes: PI, Ransom, Young (Burton et al., 1987), and breeding line N93-S-179 (N93). Breeding line N93 was selected by USDA-ARS at North Carolina State University from a cross of Young and PI. Treatments were arranged in a randomized complete block design with three replications.

Seeds for both experiments were surface-sterilized with ethanol for 1 min and 26.9 m*M* sodium hypochlorite for 3 min, rinsed six times with sterile water (Ramirez et al., 1997), and placed in the dark at 28°C in paper towels moistened with sterile 500 μM $CaSO_4$ to germinate. Five seedlings of each genotype with a tap root length of 3 ± 0.5 cm were planted in the upper compartment and thinned to four plants after 3 d. Soil moisture content was adjusted daily to 80% of container capacity (Cassel and Nielsen, 1986).

For both experiments tap root length in the subsurface compartment was measured daily. Among roots extending through the membrane, lateral and basal classes (Zobel, 1991) could not be distinguished and were combined into a group designated as "other" roots. Microbial contamination was minimized by disinfecting all tools with 20.6 *M* ethanol and by using sterilized distilled water in the subsurface compartment.

Plants were harvested 13 d after planting, which coincided with natural abscission of cotyledons and emergence of the second trifoliolate leaf. Plant shoots were dried at 55°C for 2 d, weighed, ground, dry ashed at 500°C, dissolved in 0.5 *M* HCl, and analyzed for Al and Ca by atomic absorption spectrophotometry.

Tap roots, their lateral roots, and "other" roots in the hydroponic solutions were separated at harvest and counted. Roots were refrigerated in 4.3 *M* ethanol until their length could be measured by edge discrimination (Pan and Bolton, 1991) using a desktop scanner preset to a resolution of 98 dots cm^{-1} (250 dots per inch). Roots in the soil compartment were washed free of soil and their combined total length was measured following the same procedure. Relative root length for a root class within an experiment was calculated as the ratio between a given treatment and the treatment with the greatest length.

Aluminum reacting with ferron (Bersillon et al., 1980) was measured in solution aliquots collected at Day 12 from all treatments in both experiments that contained Al. The ferron reagent was mixed with sample aliquots at a sample/reagent volume ratio of 1:3 through forced-injection into a cuvette, and absorbance readings were recorded with an automatic chart recorder from within 1 s of reagent addition until negligible changes in absorbance were observed. Matrix effects observed on ferron readings due to different Ca concentrations in solutions for Exp. 2 were taken into account by using separate standard curves for each Ca level. Two different fractions of Al, Al_a and Al_b, were characterized in the solutions using the procedure of Smith (1971). The Al_a fraction includes monomeric Al species that react almost immediately with ferron, and the Al_b fraction contains metastable Al polymers that react with ferron according to first-order kinetics. Total ferron-reactive Al (Al_T) was estimated by the following equation (Jallah and Smyth, 1998):

$$Al_T = Al_{ab} - (Al_{ab} - Al_a^0)\exp(-K_b t)$$

where Al_{ab} is the maximum value of reactive Al within the reaction time, Al_a^0 is Al_a at zero time (t), and K_b is the rate of reaction of Al_b. Nonlinear regression procedures (SAS Institute, 1985) were used to calculate Al_a, Al_b, and Al_{ab}. The GEOCHEM-PC program (Parker et al., 1995) was used to predict Al speciation in the different solution treatments.

Statistical analysis of variables measured over time was performed with a split-plot model, with the factorial combination of genotypes, Al, and pH levels as main plots, and time as the subplot. Variables measured at harvest were analyzed as a complete factorial in a randomized complete block design.

RESULTS AND DISCUSSION

Solution Aluminum Characteristics among Experiments and Treatments

Solution treatments in the subsurface compartment of both experiments affected Al speciation and ferron-reactive Al fractions (Table 1). Trivalent Al was the predominant monomeric Al species in solutions maintained at pH 4.2 and 4.6, whereas hydroxy-Al species were dominant in solutions maintained at pH 5.2. Increasing Ca concentration in solutions from 2 to 10 m*M* for Exp. 2 increased ionic strength and Ca activity and decreased the GEOCHEM-predicted activity of the sum of monomeric Al by ≈50%. Previous investigators have used the Al reacting with ferron by 30 s ($AlFN_{30}$) as an index of inorganic monomeric Al (Jardine and Zelazny, 1987; Wright et al., 1987). In solutions for both of our experiments trivalent Al activity correlated with the Al_a ($r = 0.73$, $P < 0.05$), Al_b ($r = 0.77$, $P < 0.05$) and $ALFN_{30}$ ($r = 0.83$, $P < 0.01$) fractions, but neither the predicted activities of individual hydroxy-Al species nor the sum of monomeric Al species was significantly correlated with these ferron-reactive Al fractions.

Table 1. GEOCHEM-predicted ionic strength, activities of Ca and monomeric Al species, and measured ferron-reactive Al for solution samples taken at day 12 in both experiments, averaged across genotypes.

Treatment				Predicted activities					Ferron-reactive Al		
pH	Al	Ca	I†	Ca	Al^{3+}	$Al(OH)^{2+}$	$Al(OH)_2^+$	ΣAl_{Mono}‡	Al_a§	Al_b¶	$AlFN_{30}$#
	μ*M*	mM			μ*M*						
						Exp. 1					
4.2	7.5	10	30	5.2	1.6	0.3	0.03	1.9	4.7	0.9	5.6
	15	10	30	5.2	3.2	0.5	0.06	3.8	5.8	4.8	10.6
5.2	7.5	10	30	5.2	0.8	1.2	1.50	3.5	3.4	0.6	4.0
	15	10	30	5.2	1.5	2.4	2.98	7.1	4.9	2.2	7.1
						Exp. 2					
4.6	7.5	2	6	1.4	2.7	1.1	0.3	4.1	3.7	3.3	6.6
	15	2	6	1.4	5.3	2.1	0.7	8.1	6.2	4.6	9.8
	7.5	10	30	5.2	1.4	0.6	0.2	2.2	3.0	2.9	5.2
	15	10	30	5.2	2.8	1.1	0.4	4.3	4.1	5.0	8.4

† Ionic strength.
‡ $\Sigma Al_{Mono} = Al^{3+} + Al(OH)^{2+} + Al(OH)_2^+ + Al(OH)_3^0 + Al(OH)_4^-$.
§ Al reacting instantaneously with ferron.
¶ Al reacting with ferron following first-order kinetics.
# Al reacting with ferron within 30 s.

Shoot Dry Weight and Root Length in the Soil Compartment

At harvest of Exp. 1 shoot dry weight and total root length in the limed and fertilized soil compartment were significantly ($P < 0.05$) different between genotypes PI and Ransom (Table 2). Calcium and Al treatments in the subsurface solution had no effect on shoot dry weight and total root length in the soil compartment in Exp. 2. In Exp. 2 shoot dry weight did not differ among genotypes. However, root length in the soil compartment was greater for PI than for Ransom in both experiments and similar to N93 in Experiment 2. Under field conditions PI exhibits greater drought tolerance than other genotypes (Sloane et al., 1990; Carter and Rufty, 1993), which has been attributed to extensive root proliferation in the surface soil with several orders of lateral roots (Hudak and Patterson, 1995; Pantalone et al., 1996).

Different ambient temperature and light conditions between the greenhouse in Experiment 1 and the phytotron in Experiment 2 apparently resulted in differences between experiments in shoot dry weight and root length in the soil compartment for the same genotypes (Table 2). Similarities in a given genotype's shoot biomass across treatments within each experiment support the presumption that treatment effects on root growth in the subsurface solution compartment were not confounded directly by differences in growth of plant tops.

Experiment 1

Tap Root Elongation

Tap root elongation in the subsurface solution compartment followed a curvilinear trend, but there were no significant differences ($P < 0.05$) in genotypic response to pH and Al treatments (data not shown). When averaged across pH and Al levels, tap root length for Ransom was greater than for PI ($P < 0.01$) throughout the final 6 d of growth. At harvest, tap root length for Ransom exceeded that for PI by 22%. A significant interaction ($P < 0.01$) between Al and pH revealed that tap root length, averaged across genotypes, decreased with increasing Al levels at pH 4.2 (Fig. 1). In solutions maintained at pH 5.2 there was no difference in tap root elongation between 0 and 7.5 μ*M* Al treatments, whereas root elongation stopped after Day 9 when exposed to 15 μ*M* Al. Less inhibition of tap root elongation by 15 μ*M* Al at pH 4.2 than at 5.2 may be related to the relief of Al^{3+} toxicity by increases in H^+, as recently proposed by Kinraide (1997), but Al solutions at pH 5.2 also presented conditions favorable to the formation of the rhizotoxic triskaidekaaluminium (Al_{13}) species (Kinraide, 1997). Sanzonowicz et al. (1998a) reported similar interactions between pH and Al on tap root length for Ransom in a vertically split–root experiment containing a broader range of subsurface solution treatments (pH 4.0–5.2 and 0–30 μ*M* Al).

Total Root Length and Characteristics of Lateral and Other Roots at Harvest

Aluminum concentration was the only treatment variable that significantly ($P < 0.001$) affected the total length and number of lateral roots on tap roots in the subsurface solution compartment; therefore, lateral root data (Table 3) are averaged across pH treatments. When averaged across pH levels and genotypes, coefficients of linear regression indicated that the number of lateral

Table 2. Soybean genotype shoot dry weight and total root length in the soil compartment at harvest (12-d-old plants) averaged across solution pH, Al, and Ca treatments in each experiment.

Experiment	Soybean genotype	Shoot		Root	
		Dry weight	SD	Total length	SD
		$g\ pot^{-1}$		$m\ pot^{-1}$	
1	PI	1.1	0.1	54.1	12.7
	Ransom	0.8	0.2	40.6	10.3
2	N93	0.4	0.1	32.5	8.8
	PI	0.4	0.1	39.4	13.3
	Ransom	0.3	0.1	26.6	6.3
	Young	0.4	0.1	28.0	8.8
	$LSD_{0.05}$‡			8.3	

† *F* test protected least significant difference for comparison of mean root length values for genotypes in Exp. 2. There was no significant difference in shoot dry weight among genotypes in Exp. 2.

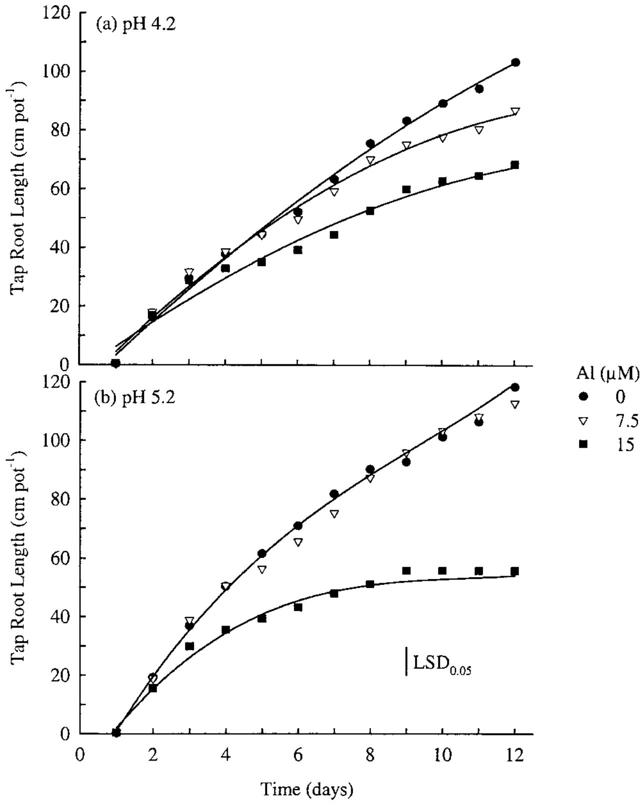

Fig. 1. Mean soybean tap root length as a function of time in subsurface solution Al treatments maintained at pH values of (a) 4.2 and (b) 5.2 in Exp. 1. Symbols are observed values averaged across genotypes Ransom and PI, and lines are predicted values from polynomial regression models. Vertical bar denotes least significant difference ($P < 0.05$) for the pH by Al by time interaction.

roots decreased by 22 per pot ($r^2 = 0.90$), and their total length decreased by 20 cm per pot ($r^2 = 0.98$) with each μM increase in Al concentration. The average length of a lateral root decreased by 0.02 cm ($r^2 = 0.98$) and the density of lateral roots on tap roots decreased by 0.08 roots cm^{-1} ($r^2 = 0.98$) with each μM increase in solution Al concentration. The average length of a lateral root was greater for Ransom than for PI. Since the number of lateral roots was similar among genotypes, the greater density of lateral roots on tap roots for PI was related to a shorter length of tap roots relative to Ransom.

The total length of other roots crossing the membrane from the soil compartment at harvest was significantly ($P < 0.01$) greater for Ransom than for PI. Mean total length of the other root class, averaged across pH and Al treatments, was 126 cm per pot for Ransom and 75 cm per pot for PI. The number of other roots in the subsurface compartment was similar for both genotypes and was not affected by solution pH and Al treatments. Consequently, the average length of a root in this category was longer for Ransom than for PI (Table 3). Inspection of root systems at harvest indicated that most of the roots extending into the solution from the soil compartment were basal roots according to the nomenclature used by Zobel (1991).

The combined length of all root classes in the solution compartment at harvest was significantly greater ($P < 0.01$) for Ransom than for PI. Total root length for Ransom exceeded that of PI by 22% when averaged across Al and pH treatments. Although there were no differences in genotypic response to solution Al and pH treatments, reductions in total root length with increasing solution Al concentration differed between solutions at pH 4.2 and 5.2, when averaged across genotypes (Fig. 2). Inhibition of total root length by the 7.5 μM Al treatment was greater in solutions maintained at pH 4.2 than at 5.2, whereas inhibition of root length with 15 μM Al was similar at both pH levels.

In absence of Al, lateral roots were the dominant component of total root length in both genotypes and at both pH levels (Fig. 2). Inhibition of elongation by Al among root classes was proportionately greater for

Table 3. Measured and calculated parameters for the lateral and the "other" root class in the subsurface solution compartment among soybean genotypes and solution Al treatments at harvest of Exp. 1, averaged across solution pH values of 4.2 and 5.2.

Soybean genotype	Solution Al	Lateral root: Number	Lateral root: Length, Total	Lateral root: Length, Mean†	Lateral root: Density‡	Other root mean length
	μM	roots pot^{-1}	cm pot^{-1}	cm root^{-1}	number cm^{-1}	cm root^{-1}
PI	0	566	363	0.7	5.7	1.7
	7.5	474	159	0.4	5.1	1.7
	15	214	43	0.2	4.8	1.1
	Mean	418	188	0.4	5.2	1.5
Ransom	0	528	353	0.7	4.4	2.6
	7.5	419	205	0.5	3.9	3.5
	15	229	76	0.4	3.0	2.0
	Mean	392	211	0.5	3.8	2.7
				Al Means		
	0	547	358	0.7	5.1	2.2
	7.5	447	182	0.5	4.5	2.6
	15	222	60	0.3	3.9	1.6
LSD$_{0.05}$:						
Genotype		NS§	NS	0.1	0.5	0.7
Al		25	17	0.1	0.6	NS
Genotype × Al		NS	NS	NS	NS	NS

† Total length/number of roots for each respective root class.
‡ Number of lateral roots/length of tap root.
§ Denotes nonsignificant ($P < 0.05$) F test.

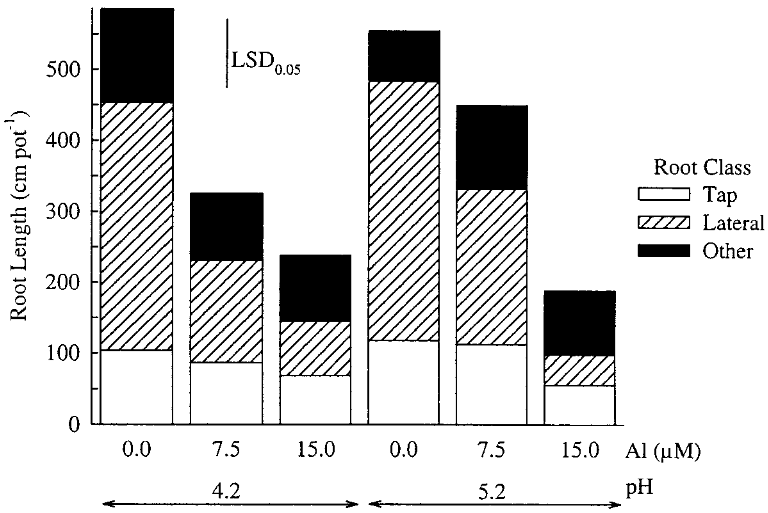

Fig. 2. Total soybean root length and length of individual root classes at harvest in subsurface solution pH and Al treatments in Exp. 1. Root length is averaged across genotypes Ransom and PI. Vertical bar denotes the least significant difference ($P < 0.05$) for total root length.

laterals than for tap and other roots. When averaged across genotypes and pH treatments, lateral root length with 15 μM Al was 17% of that in solutions without Al, compared with 56% for tap roots and 90% for other roots. In previous vertically split–root experiments with Ransom, Sanzonowicz et al. (1998a) observed that both Al and H^+ inhibited lateral root elongation to a greater extent than tap roots.

In separate experiments comparing tap root elongation among soybean genotypes upon exposure to Al in single-compartment hydroponic systems, investigators ranked PI as Al-tolerant (Villagarcia et al., 1997) and Ransom as Al-sensitive (Sartain and Kamprath, 1978). However, neither of these investigations contained both genotypes. Although our direct comparisons of these genotypes in a vertically split–root system revealed that PI had greater root length in the limed soil compartment, there were no differences in genotypic responses to Al and H^+ inhibition of either the combined or individual elongation of several root classes in the subsurface solution compartment.

Experiment 2

Tap Root Length and Characteristics of Laterals and Other Roots at Harvest

During 12 d of exposure to subsurface solution Al and Ca treatments, tap root elongation of soybean genotypes N93, PI, Ransom, and Young followed curvilinear trends similar to those shown in Fig. 1 for PI and Ransom in Exp. 1 (data not shown). Likewise, differences in tap root elongation among genotypes to solution treatments were reflected in root length measurements taken at harvest. Tap root length at harvest was significantly different ($P < 0.05$) among genotypes when averaged across solution Al and Ca treatments. Genotypic

Table 4. Effect of solution Al and Ca concentration in the subsurface compartment on tap root length of four soybean genotypes at harvest of Exp. 2.

		Genotypes				
Al	Ca	N93	PI	Ransom	Young	Mean
μ*M*	m*M*	Tap root length, cm pot^{-1}				
7.5	2	38.9	41.0	39.7	38.3	39.5
	10	76.0	90.6	121.6	117.6	99.6
	Mean	57.5	65.8	72.4	77.9	68.2
15	2	20.7	10.9	12.0	11.4	13.8
	10	65.9	51.4	107.7	77.8	75.7
	Mean	43.3	31.2	59.9	44.6	44.7
		Ca treatment means				
	2	29.8	26.0	25.8	24.9	26.6
	10	71.0	71.0	113.3	97.7	87.1
		Genotype means				
		50.4	48.5	65.6	61.3	
$LSD_{0.05}$:						
Al		2.6				
Ca		2.6				
Al × Ca		NS†				
Genotype		3.6				
Genotype × Al		NS				
Genotype × Ca		5.1				
Genotype × Al × Ca		NS				

† Denotes nonsignificant ($P < 0.05$) *F* test.

Table 5. Mean lateral root length at harvest averaged across four soybean genotypes for subsurface solution Al and Ca treatments in Exp. 2.

Al	Ca (mM) 2	10	Mean
μM	Lateral root length, cm pot^{-1}		
7.5	21	108	64
15	11	40	25
Mean	16	74	
$LSD_{0.05}$:			
Al		13	
Ca		13	
Al × Ca		18	

ranking for tap root length was: Ransom > Young > N93 = PI (Table 4). When comparing main effects of solution Al and Ca treatments, tap root length decreased by 34% between 7.5 and 15 μM Al and increased by threefold between 2 and 10 mM Ca. Inhibition of tap root length by Al among genotypes was influenced by the solution Ca concentration. Tap root length with 2 mM Ca, averaged across Al treatments, was similar among genotypes (mean of 27 cm per pot), but tap root length for cultivars Ransom and Young was greater than for line N93 and PI when solutions contained 10 mM Ca. These genotypic differences in tap root elongation for Al treatments with 10 mM Ca were only evident after 9 d of root exposure to the subsurface solutions (data not shown). Relief of Al inhibition of soybean tap root elongation upon increasing solution Ca concentration has been reported for several genotypes in experiments with both single-compartment (Alva et al., 1986b; Noble et al., 1988) and vertically split–root systems (Lund, 1970; Sanzonowicz et al., 1998b).

Total length of laterals on tap roots at harvest was significantly affected by solution concentrations of both Al and Ca, but there were no differences ($P < 0.05$) between genotypes. For solutions with 2 mM Ca and either 7.5 or 15 μM Al lateral root lengths averaged across genotypes were not significantly different, whereas lateral root length in solutions with 10 mM Ca decreased by 63% between solutions with 7.5 or 15 μM Al (Table 5). Amelioration of Al inhibition of lateral

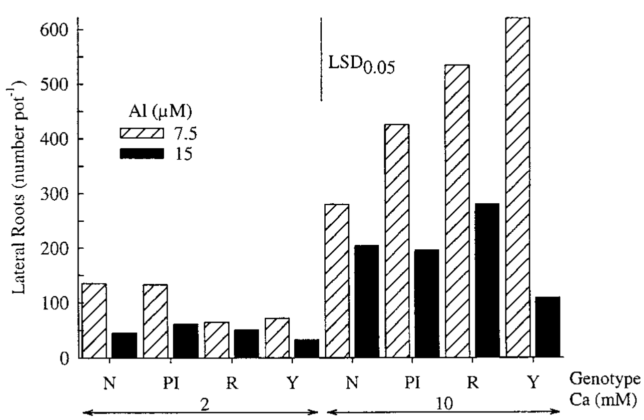

Fig. 3. Number of lateral roots on tap roots at harvest in the subsurface compartment for soybean genotypes N93 (N), Ransom (R), PI, and Young (Y), as a function of Al concentration in solutions with 2 and 10 mM Ca in Exp. 2. Vertical bar denotes the least significant difference ($P < 0.05$) for the genotype × Al × Ca interaction.

Table 6. Estimated density of lateral roots on tap roots at harvest in the subsurface solution compartment based on mean values for the number of lateral roots and length of tap roots in each soybean genotype, Al, and Ca treatment combination in Exp. 2.

Al	Ca	Genotype N93	PI	Ransom	Young	Mean
μM	mM	Lateral root density on tap roots, number cm^{-1}				
7.5	2	3.5	3.2	1.6	1.9	2.6
	10	3.7	4.7	4.4	5.3	4.5
	Mean	3.6	4.0	3.0	3.6	
15	2	2.2	5.7	4.3	2.9	3.8
	10	3.1	3.8	2.6	1.4	2.7
	Mean	2.7	4.8	3.5	2.2	
		Ca treatment means				
	2	2.9	4.5	3.0	2.4	3.2
	10	3.4	4.3	3.5	3.4	3.7
		Genotype means				
		3.1	4.4	3.2	2.9	

root elongation by increasing Ca supply from 2 to 10 mM was proportionately greater in solutions with 7.5 than with 15 μM Al.

There was a significant interaction ($P < 0.01$) among genotypes and Al and Ca treatments on the total number of lateral roots on tap roots at harvest. In solutions containing 2 mM Ca, there was no difference in the number of lateral roots among genotypes exposed to solutions with either 7.5 or 15 μM Al (Fig. 3). In treatments with 10 mM Ca, increasing Al concentration from 7.5 to 15 μM Al decreased the number of lateral roots by 48% for Ransom, 54% for PI, and 82% for Young, whereas there was no significant difference in the number of lateral roots between Al treatments for line N93.

There was a correlation ($r = 0.98$) between the total number and length of lateral roots across all 16 treatment combinations of genotype, Al, and Ca. Thus, the length of a lateral root was relatively constant among genotypes as well as Al and Ca treatments. Based on the linear regression between total number and length of lateral roots, the average length of a lateral root in this experiment was 0.23 cm. Since the length of a lateral root remained constant among genotypes and solution treatments, observed differences in total lateral root length among Al and Ca treatments (Table 5) resulted from a combination of differences in tap root length (Table 4) and the number of these laterals per unit length of tap root (lateral root density). The density of laterals on tap roots, shown in Table 6, was calculated from treatment mean values for the number of laterals and the length of tap roots. Mean values across Al and Ca treatments indicate that lateral root density for PI was greater than for other genotypes. Changes in lateral root density upon exposure to increasing solution concentrations of Al or Ca were not consistent among genotypes. Mean values of lateral root density averaged across Ca treatments for line N93 and Young decreased with increasing Al concentration, but increased for PI and Ransom. For similar comparisons between 2 and 10 mM Ca averaged across Al treatments, lateral root density increased with Ca concentration for line N93, Ransom, and Young but was almost constant for PI. Collective results for these root characteristics indicate

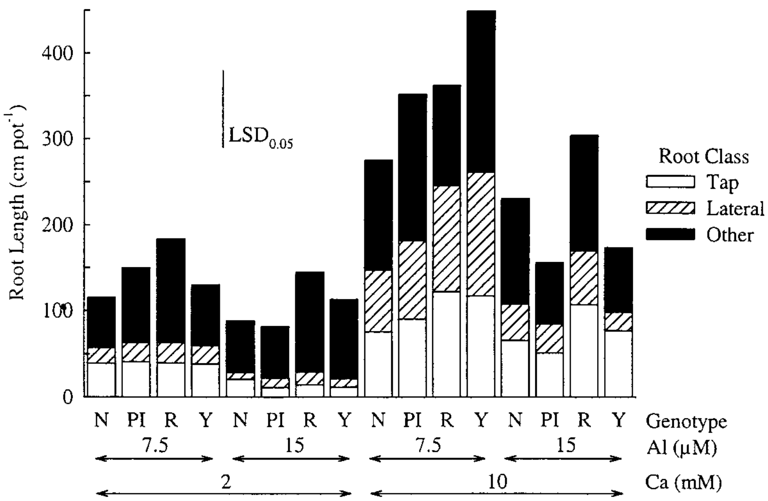

Fig. 4. Total root length at harvest as a function of root classes and solution Al and Ca treatments in the subsurface compartment for soybean genotypes N93 (N), Ransom (R), PI, and Young (Y) in Exp. 2. Vertical bar denotes least significant difference ($P < 0.05$) for the genotype × Al × Ca interaction.

that differences in total lateral root length among solution Al and Ca treatments cannot be solely attributed to a multiplier effect from changes in tap root elongation.

Significant differences ($P < 0.05$) in the total length of other roots extending from the soil into the subsurface solution compartment were restricted to the main effect of solution Ca concentration. Between 2 and 10 m*M* Ca, total length for this root class, averaged across genotypes and Al treatments, increased from 84 to 126 cm per pot. The number of other roots extending into the solution compartment was similar between genotypes (mean values ranging from 36 to 44 per pot) but differed between solution Al and Ca treatments. A significant Ca × Al interaction was associated with a greater number of these roots (55 per pot) in solutions with 15 μ*M* Al and 2 m*M* Ca than in the other treatments (mean value of 34 per pot). These results are consistent with previous observations by Sanzonowicz et al. (1998b) that subsurface solution Al and Ca concentrations that inhibited tap and lateral root elongation for Ransom increased the number of other roots that extended from the soil into the solution compartment.

Total Root Length at Harvest

Genotypic differences in the combined length of tap, lateral, and other roots in the subsurface solution compartment depended on the solution Al and Ca treatments (Fig. 4). In solutions containing 2 m*M* Ca, total root length of soybean genotypes was similar between treatments containing either 7.5 or 15 μ*M* Al. Increasing Al concentration in solutions with 10 m*M* Ca decreased total root length by 56% for PI and 61% for Young, but there was no significant change for line N93 and Ransom. Genotypic ranking for total root length in solutions with 10 m*M* Ca also differed between Al treatments. At 7.5 μ*M* Al total root length of Young was greater than for line N93 and PI; whereas at 15 μ*M* Al total root length for Ransom was greater than that of PI and Young. Among the different root classes, the greatest reduction in length in response to increased Al stress at 10 m*M* Ca occurred with lateral roots; between 7.5 and 15 μ*M* Al, reductions in length of root classes averaged across genotypes were 63% for lateral roots, 26% for tap roots, and 33% for other roots.

Aluminum and Calcium Accumulation in Soybean Shoots

Despite similar shoot dry weights among genotypes and solution treatments at harvest (Table 2), Al accumulation in shoots decreased with increasing Ca concentration in the subsurface solution compartment (Table 7). There were also genotypic differences in Al accumulation in shoots when averaged across solution Al and Ca treatments for the subsurface root compartment. Aluminum accumulation for PI was greater than for Young and line N93, and similar to that for Ransom. Decreasing Al accumulation in soybean shoots with increased Ca concentration in subsurface solution Al treatments would be consistent with the findings of Kinraide and Parker (1987) that competition by Ca reduces access by Al to external binding sites at the plasmalema. Significant differences ($P < 0.05$) in Ca accumulation in soybean shoots were restricted to an increase from 4 to 5 mg per pot between solutions with 2 and 10

Table 7. Aluminum accumulation at harvest in aboveground biomass of four soybean genotypes in Exp. 2 as influenced by Ca concentrations in the subsurface solution compartment averaged across solution treatments of 7.5 and 15 μ*M* Al.

Solution Ca treatment	Genotype				
	N93	PI	Ransom	Young	Mean
	shoot Al, μg pot^{-1}				
2	17	30	24	20	23
10	17	19	19	14	17
Mean	17	25	22	17	
LSD$_{0.05}$:					
Ca			4		
Genotype			6		
Ca × Genotype			NS†		

† Denotes nonsignificant ($P < 0.05$) *F* test.

Table 8. Prediction equations for relative length of tap and lateral soybean roots in the subsurface solution compartment of Exp. 2, based on molar activity ratios between Ca and monomeric Al species.

Variables				
Dependent	Independent	Genotype	Equation	R^2
Tap root	$\{Ca/Al^{3+}\}$	All	$Y = 107 - 106\exp(-0.0007X)$	0.98
	$\{Ca/\Sigma Al_{Mono}\}$	All	$Y = 106 - 106\exp(-0.001X)$	0.98
Lateral root	$Ca/Al^{3+}\}$	N93	$Y = 192 - 189\exp(-0.0002X)$	0.99
		PI	$Y = 1.3 + 13\exp(0.0006X)$	0.99
		Ransom	$Y = 4.2 + 0.03X$	0.99
		Young	$Y = 9.9 + 0.3\exp(0.002X)$	0.99
	$\{Ca/\Sigma Al_{Mono}\}$	N93	$Y = 189 - 186\exp(-0.0003X)$	0.99
		PI	$Y = 1.4 + 13\exp(0.0006X)$	0.99
		Ransom	$Y = 4.3 + 0.04X$	0.99
		Young	$Y = 9.8 + 0.3\exp(0.02X)$	0.99

mM Ca when averaged across genotypes and solution Al treatments.

Relations between Root Length and Solution Aluminum/Calcium Ratios

Alleviation of Al rhizotoxicity by increased molar activity of Ca in hydroponic solutions has been reported for several plant species including soybean (Rengel, 1992). Runge and Rode (1991) proposed that the degree of Al rhizotoxicity depends more on Ca/Al molar activity ratios than on their individual activities in solution. Genotypic differences in Ca alleviation of the inhibitory effects of Al on elongation of tap and lateral roots were further investigated by relating length at harvest of each root class to ratios of GEOCHEM-predicted molar activities of Ca and either individual monomeric Al species or their sums (Table 1). Regression equations for the best linear and nonlinear relations are shown in Table 8. Relative tap root length for all genotypes was predicted by a single equation, but there were significant interactions ($P < 0.05$) between the Ca/Al ratios and genotypes for relative length of lateral roots. Regressions with both the $\{Ca/Al^{3+}\}$and the $\{Ca/\Sigma Al_{Mono}\}$ ratios explained between 98 and 99% of the variability in relative length of tap and lateral roots.

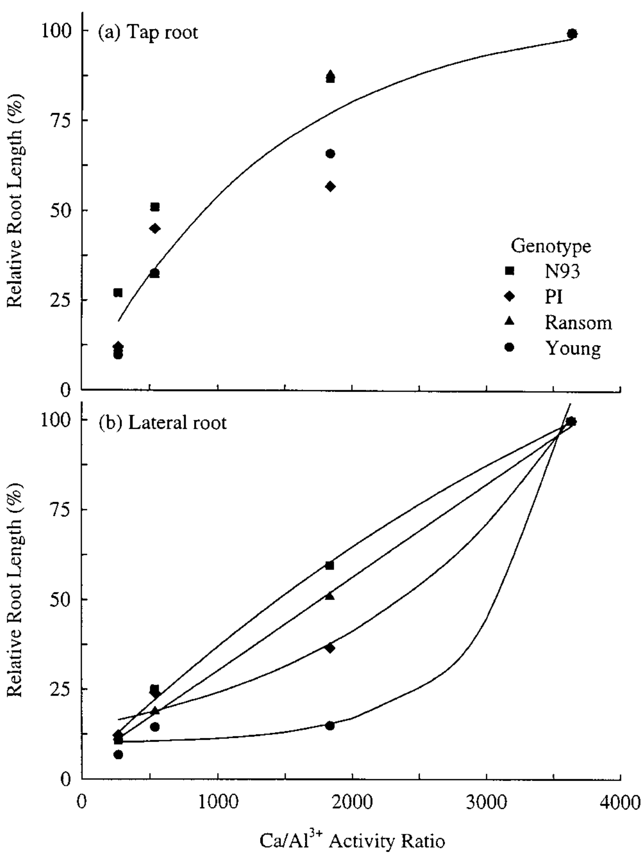

Fig. 5. Observed (symbols) and predicted (lines) relative length at harvest of (a) tap roots and (b) lateral roots of soybean genotypes N93, Ransom, PI, and Young in the subsurface solution compartment as a function of the molar activity ratios between Ca and Al^{3+} in Exp. 2. Prediction equations are shown in Table 8.

Relations between relative root length and the $\{Ca/Al^{3+}\}$ ratio are shown in Fig. 5 for both tap and lateral roots. A 50% reduction in relative tap root length occurred with a $\{Ca/Al^{3+}\}$ ratio of 891 for all genotypes. Relative lateral root length increased exponentially with increasing $\{Ca/Al^{3+}\}$ ratio for line N93, PI, and Young, and linearly for Ransom. A 50% reduction in relative length of lateral roots occurred with $\{Ca/Al^{3+}\}$ ratios of 1445 for line N93, 1527 for Ransom, 2355 for PI, and 3088 for Young. This means that lateral roots required a greater concentration of solution Ca than tap roots to alleviate rhizotoxic effects of a given Al concentration. Furthermore, line N93 and Ransom achieved a given level of relative lateral root length in Al-toxic solutions with less Ca than PI and Young.

Simple ionic compositions of subsurface compartment solutions (Table 1) favored the use of $\{Ca/Al^{3+}\}$ as an index for root elongation among genotypes and root classes in our experiments. In soil systems or more complex nutrient solutions, ameliorative effects of other cations besides Ca must also be considered. With wheat (*Triticum aestivum* L.) seedlings, for example, Kinraide et al. (1994) reported that Ca, Mg, and Sr were equally effective in ameliorating Al inhibition of root elongation.

CONCLUSIONS

By simulating acidity conditions underlying a limed surface soil, the vertically split–root system used in these experiments allows assessment of root elongation responses to H^+, Al, and Ca with minimum confounding effects on shoot growth. The only difference in tap root elongation among genotypes to subsurface solution treatments was for the mean value across Al treatments with 10 mM Ca; lengths for Ransom and Young were greater than for line N93 and PI. However, in both experiments, mean values of tap root length averaged across solution treatments for Ransom were greater than for the other genotypes tested.

Hydrogen and Al inhibited the length of lateral roots to a greater extent than tap roots. Alleviation of the inhibitory effect of Al on root length by increasing Ca supply in solutions also required greater Ca concentrations for laterals than for tap roots. Expression of genotypic differences in lateral root length to rhizotoxic Al was influenced by the associated level of Ca supplied in the solutions. On the basis of the relative length of lateral roots across solutions with varying molar activity ratios between Ca and Al^{3+}, Ransom and line N93 had greater tolerance rankings than Young and PI. Based on the absolute total length of roots in the subsurface compartment, similar genotypic rankings for Al tolerance were obtained in the treatment with 15 μM Al and 10 mM Ca; total root length for Ransom was similar to line N93 and greater than that for PI and Young. The observed differences among genotypes in tap and lateral root length upon exposure to solution Al illustrate the importance of characterizing individual root classes when screening soybean germplasm for Al tolerance.

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